

REPAIR AND REATTACHMENT IN THE BALANIDAE AS RELATED TO THEIR CEMENTING MECHANISM

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An investigation of the attachment of sessile Cirripedia was initiated to aid future studies on fouling problems. The primary objective was the collection of the barnacle adhesive secretion, generally called the cement, for analysis and tests. The observation that detached or injured barnacles produce a cement-like secretion directed the course of the investigation into a study of the anatomy and functions of the cementing organs to determine the nature of this healing secretion.

The young barnacle begins to grow soon after permanent attachment and metamorphosis have been completed. As is characteristic of the phylum Arthropoda, the body of the barnacle grows in well-defined periods distinguished by the moulting of the exoskeleton. The shell of the barnacle also undergoes cyclic growth (Darwin, 1854) as evidenced by striations on various parts of the shell: the basis, the opercular valves and the conical portion of the shell composed of plates, for which the term "shell wall" is used here, for simplicity. Of particular interest are the striations on the basis, which form concentric circles and permanently record the basis perimeters of previous growth cycles. Darwin (1854) believed that the periods of shell growth are closely related to the moulting cycles. Our histological studies on the development of the cement ducts (Saroyan, Lindner and Dooley, 1968) seem to support the correlation between growth of the basis and moulting. This study shows that the cement duct network is part of a cuticular membrane and is secreted by epithelium cells. At each moulting, this membrane and the duct network are also moulted but become trapped inside the shell. Since each duct network leads to the basis perimeter at that particular time, the relationship between moulting and growth of the basis is strongly indicated. Costlow and Bookhout (1953, 1956, 1957) and Costlow (1956, 1959), conducted extensive studies on the shell growth of *Balanus amphitrite* and *B. improvisus*; and the elaborate experiments designed particularly to explore the connection between shell growth and moulting indicated no direct correlation. They showed that the shell grows continually, though erratically and independently from the moulting (Costlow and Bookhout, 1953, 1956; Costlow 1956, 1959). These findings were based on the outside measurements of the basal perimeter, which also partially include the thickness of the wall plates at the basal edge. Since the perimeter of the basis is overlapped by the edges of the wall, the growth rate of the basis and its relation to the moulting period is not entirely understood.

During growth the leading edges of the calcareous shell grow outward; thus, the basis and the basal margin of the shell walls become larger in diameter and the shell walls are pushed upward. The shell walls and the basis are tightly connected by numerous muscle tissues around the basal perimeter at the inside joints of the

leading edges. The contraction of these muscles pull the basis upwards and at the same time the shell wall downwards. Since the basis is usually firmly cemented to the substratum, the wall is pulled down and presses the basal edge of the shell wall tightly down on the substratum. The shell wall rests upon the growing edge of the basis, which is a thin, flexible membrane of chitin, regardless of whether or not the species under examination has a calcareous basis (Costlow, 1956; Newman, Zullo, and Wainwright, 1967). Consequently, this thin flexible rim of the basis is also pressed very tightly to the substratum leaving only a very slight gap. Due to this peripheral pressure, the basis may grow into the recessions and over the irregularities of a solid substratum, faithfully duplicating its surface structure (Pilsbry, 1916; Gregg, 1948) and obtaining maximum contact area for adhesion. The forces produced by the growing barnacle enable it to plow away loose deposits, fouling organisms, and detritus or to dig beneath soft materials, such as clay or certain coatings (Bärenfänger, 1939; Harris, 1946) in order to reach an underlying solid surface. The barnacle cements itself to the substratum by an adhesive which is secreted at the perimeter of the basis and spreads under it to fill any gap between basis and substratum (Darwin, 1854). Due to the peripheral pressure exerted by the barnacle, the gap to be filled and, therefore, the thickness of the cement layer, is normally less than five microns.

In crowded communities, however, the barnacles may develop abnormally. For example, in such communities *Balanus balanoides* grows into elongated shapes, and Darwin (1854, page 147) observed that sometimes only the shell walls of such specimens reach the substratum, while the noncalcareous basal membrane remains suspended and deeply concave. Darwin found that "thickish roots" were hanging from the basal membrane in the resulting gap. He believed these roots to be cement.

This development form apparently escaped the interest of later investigators since only a few references can be found in the literature on this subject. Crisp (1960) describes specimens which survived complete upward displacement by neighboring barnacles but does not mention any adhesive secretion. Crisp also found that specimens of *Balanus balanoides* have some limited mobility under lateral pressure by neighboring barnacles and can be moved along the surface of a smooth substratum several centimeters away from the original point of attachment. He speculated that the advancing edges form new adhesions as the barnacle gradually undergoes lateral displacement. In a recent article (Newman, Zullo, and Wainwright, 1967, page 170) there is a reference to some unpublished observations, "that *Balanus(B) a. amphitrite* Darwin if carefully removed from the substratum without noticeably damaging the wall or calcareous basis, could reattach itself to glass slides, by first cementing and then calcifying itself in place. This is accomplished by protrusion of portions of the mantle from the small spaces that normally occur along the seam between the wall plates and the basis." This short reference, however, neither takes the cementing apparatus into consideration nor goes into detailed explanation of the mechanism of the reattachment process.

The basic anatomy of the cementing apparatus of the Balanidae was described by Darwin (1854). Darwin describes a series of complicated duct networks in a number of species, including *Balanus tintinnabulum*. Each network originates from a pair of pear-shaped vesicles and after much branching and occasionally

rejoining the ducts lead to and terminate in orifices around the basal perimeter or, as in the case of older networks, around the concentric circle which marked the earlier growth cycle. The vesicles are also connected by a channel and form a pair of chains resembling strung beads. Darwin believed that the cementing apparatus is a modified part of the reproductive system and that these vesicles are the remains of degenerated cement glands. Not much later, Krohn (1859) recognized the cementing apparatus as a separate organ, and expressed his doubt about the vesicles being secretory glands. Although some additional studies (Pagenstecher, 1863; Gruvel, 1905; and Thomas, 1944) contributed somewhat to the concept of the cementing apparatus of the Cirripedia, no cement glands of any species of the Balanidae were described until quite recently. Lacombe (1966, 1967, 1968) described the cement gland cells and the cementing apparatus of *Balanus tintinnabulum*, although the description of the duct network deviates somewhat from the accurate description of that of the same species by Darwin (1854) and does not mention the vesicles or their function. While Lacombe indicates that each separate duct network of *B. tintinnabulum* develops its own cement gland cells, suggesting a temporary glandular function, in a previous paper (Saroyan, Lindner, and Dooley, 1968), we demonstrated that the gland cells in several other *Balanus* species develop from the cyprid cement glands independently of the duct network; and the same glands function throughout the whole life of the adult barnacle. We also showed that the cement gland cells, although largely intertwined with the ovarian tissues, are more abundant at the lateral area of the mantle, around the end of the two main channels, which rise from the basis into the mantle tissue in close proximity to the lateral scutal depressor muscle. We demonstrated that the cementing apparatus of these *Balanus* species is basically identical to that of the Lepadidae, the other family of Cirripedia, namely in having periodically functioning permanent glands on both sides of the mantle; cement glands consisting of smaller units, which are connected by ducts at a node, where the chemical properties of the secretion are altered; and only one pair of main channels, which conduct the secretion from the node toward the initial attachment point, where the remains of the cyprid antennules can be found. In the Balanidae, a pair of vesicles form around the basal portion of the main channel in each growing period, thus the main channel runs through the vesicles as a continuous tube (Fig. 20). The cement enters the vesicle through the permeable walls of the main channel portion contained within the vesicle. Under normal conditions, the cement does not follow the main channel beyond the newest vesicle toward the initial attachment point and does not reach the older vesicles. At the newest vesicle, which is usually the closest to the basis perimeter, the cement changes its course to find its way through the duct network to the perimeter. As we showed through the histology of their development, the vesicles do not have any secretory function; their purpose is mainly the distribution of the cement into the ducts.

We also found (Saroyan, Lindner, and Dooley, unpublished) that the vesicles may be capable of a pumping action and therefore may be responsible for the transportation of the cement. This pumping action is based upon the barnacle's capability of increasing the pressure inside the mantle cavity by contracting the depressor muscles. As the closed valves depress, they also compress the seawater in the mantle cavity. This pressure increase of the water is transmitted through

the resilient walls of the vesicle to its contents as the vesicle is compressed. The soft, flexible walls of the main channel inside the vesicle collapse from the pressure and shut off the passage back in the main channel. The content of the vesicles therefore is forced into the ducts. When the pressure in the mantle cavity is released, the resilient vesicle regains its original shape and volume, drawing more fluid in. By size considerations, there is less resistancy in the main channel than in the ducts, therefore the additional fluid will be drawn into the vesicle from the main channel rather than back from the ducts. With such repeated cycles, the secretion can be forced through the duct network quite rapidly with the vesicle serving as a combination of distributing chamber, pump, and checkvalve.

METHODS

Attached adult *Balanus crenatus*, *B. glandula*, and *B. cariosus* from Point Reyes, California, and *B. improvisus* from Mare Island Strait, California, were collected on Plexiglas and rubber panels in natural environments. Specimens were removed intact from these panels and successfully reattached on glass microscope slides in the laboratory.

Adult barnacles of *Balanus crenatus*, *B. glandula*, and *B. improvisus* were also collected on glass microslides in natural environments for microscopic study of the basis and attachment. The specimens were killed, fixed, decalcified, and stained on the original slides. Ten per cent neutral buffered formalin by Lillie (1954) (Pearse, 1961), Baker's (1944) formol-calcium (Pearse, 1961), and Zenker's fixative (Gray, 1954) were used for fixation. Specimens fixed in Baker's formol-calcium for 24 hours, decalcified in Jenkin's fluid (Pearse, 1961) for 24 hours, and refixed in Baker's formol-calcium for 48 hours provided the most satisfactory results.

For studying barnacles in microtome sections, solid paraffin blocks were exposed at the fouling sites. Barnacles attached to the paraffin blocks were allowed to reach sizes up to 6 mm in diameter before they were fixed, then decalcified while still attached to the paraffin substratum. During the later steps of dehydration, the substratum was dissolved to leave the specimen with all substances between its basis and the substratum intact for embedding and sectioning. These specimens were embedded in Paraplast and cut into 10-micron sections.

For general histology, Mallory's Trichrome was adapted but some additional staining techniques, such as Eosin-Aniline blue and PAS, were also used. For the Mallory Trichrome technique, a shortening of the usual staining and rinsing times (Gray, 1954) was necessary to obtain good results with arthropod tissues. This technique consisted of immersing the preparation for one minute in a one per cent Acid Fuchsin solution, one minute in one per cent phosphotungstic acid, and two minutes in a solution containing two per cent Orange G, two per cent oxalic acid, and 0.5 per cent Aniline blue WS. The Acid Fuchsin and the phosphotungstic acid solutions each were followed by a one minute rinse, and the final solution by a five minute rinse in distilled water. The preparation was then dipped briefly in 95 per cent alcohol (15 sec) and then put through two absolute alcohol rinses of three minutes each. Two five minute rinses in two changes of xylene preceded the final mounting in Permount.

RESULTS

During the collection of large specimens of *Balanus nubilus*, one of the barnacles suffered a sizable crack in its basis. After 24 hours, an abundant white, opaque, rubbery exudate, or secondary secretion, was found which filled and sealed this injury (Fig. 1). On numerous occasions it has been observed that barnacles sustain injury such as cracks or breaks in their calcareous bases as a result of the forces produced either by themselves or their neighbors. If vital organs are not seriously damaged or if the injury is not too extensive, the barnacle may survive such an accident by repairing the injuries with these secretions. Microscope preparations show that secretions around or in the cracks of the basis have staining characteristics similar to those of the adhesive cement of the barnacles (Figs. 2 and 3).

Secondary secretions were found not only in injured barnacles, but also in specimens of *Balanus crenatus* and *B. glandula* that were partially or completely separated from the substratum. Some of these separations appear to have been caused by excessive force exerted by the barnacle itself in an effort to press the growing edges of the basis close to the substratum. The pressures created at the perimeter may result in lifting and detaching the central portion of the basis. Such bases become concave and the ensuing gap is usually filled with the secondary secretion (Fig. 4).

Microtome sections of these thick layers of secretions often have a cavernous and vertically striated appearance (Fig. 5). This effect is probably caused by the continuous recession of the basis from the substratum. The gap created between basis and substratum is filled with the fluid secretion, but before hardening can take place, the basis continues to recede. The already viscous secretion may then pull threads of material between the two surfaces, thus creating a loose structure. These new gaps are then filled with fresh secretions during the next period; and so the process is continued until the recession either ceases or continues at so rapid a rate that the secretory system is no longer able to supply enough material to fill the gap.

Another type of separation can occur in gregarious communities where the shell walls of neighboring barnacles may be fused together. Since the shell walls grow up from their bases, a faster growing specimen may lift up and detach a slower growing one from the substratum (Fig. 6). The space between the elevated barnacle and the substratum is usually then filled by the opaque secretion. If the gap is too large to be filled, any secretion present may be seen hanging from the basis, indicating that an effort was made by the barnacle to reach the lost substratum to reattach. The rubbery secretion often covers the whole basis in several millimeter thick layers (Fig. 7) concealing the fine structure of the calcareous basal surface. This fine structure exhibiting the well distinguished cement duct network can be seen on the basis of carefully detached specimens such as the ones used in our reattachment experiments (Fig. 8). These barnacles were removed intact from smooth test panels and subsequently reattached to other smooth surfaces, such as glass microscope slides. Successful reattachments were observed in specimens regardless of whether they were totally submersed in seawater or suspended in such a way that only the opercular region was submersed with the



FIG. 1



FIG. 4



FIG. 2

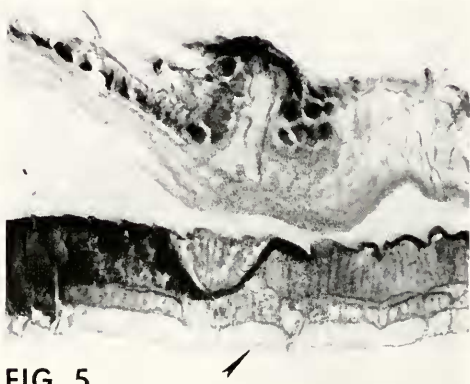


FIG. 5



FIG. 3



FIG. 6

FIGURE 1. Large specimen of *Balanus nubilus* species suffered a crack in its basis. Within 24 hours, the secretion seen here hardened and repaired the crack.

FIGURE 2. Dark, stained cement in vicinity of (A) circular crack in basis of attached adult barnacle, near (B) initial attachment patch (Wholemound, Masson-Patay, 40 $\times$).

FIGURE 3. Section through basis of attached adult *Balanus crenatus* showing crack in basis filled with cement (10- μ section, Mallory's Trichrome, 180 $\times$).

FIGURE 4. Abnormal concave basis on *Balanus crenatus* specimen detached from solid substratum in the laboratory shows abundant secretion, which represents an attempt by the barnacle to fill the central gap between basis and substratum.

basis and the new substratum out of contact with the seawater. The reattachments were accomplished by the white opaque substance secreted on the basal surface and spread between basis and substratum (Fig. 9). The closer the contact of these two surfaces, and hence the thinner the secretion layer, the firmer the reattachment appears to be. The reattachment can be so strong that the shell walls and body will break away before the basis can be detached. If the intervening space between basis and substratum is too large to be filled, thick droplets of secretion appear and hang from the basis (Fig. 10). These reattached specimens can be kept alive indefinitely with proper care and seem to develop normally.

Assuming that the secretion is cement, it could be expected that the secretion would originate from the perimeter of the basis where the newly developed cement duct orifices are located. In general, the secretion does appear at the perimeter, from which it then spreads under the separated or injured area, but centers of secretions can be detected inside the perimeter also (Figs. 9 and 10). In addition, occasionally, an extensive separation or injury occurs isolated within the perimeter and the secretion from the perimeter cannot reach the affected areas in sufficient quantities. Indications are that the barnacle is able to grow new, irregular cement ducts into such a damaged area (Fig. 11). These emergency or secondary cement ducts are larger in diameter than the normal ducts and have few or no bifurcations. Such a duct extends directly from the newest formed vesicle to the damaged area where it ends in an orifice. Normally, only the first vesicle formed after metamorphosis would have such a duct leading from it, as with each succeeding growth period, the duct system becomes increasingly complex in the number of branchings before the final orifices are reached.

The secondary cement ducts usually can be found only in those detached or injured areas where no old primary ducts and orifices can be found as, for example, near the cyprid attachment where the first available duct ends are outside the perimeter of the innermost circle (Fig. 12). There is probably a unique mechanism that enables the barnacle to recognize the need for new ducts and to initiate the growth of these unusual ducts. Likewise, the dissolution of the calcareous matter to permit the growth of new ducts into old sites must somehow occur; similar processes are known in nature.

In the majority of cases, however, where detachment involves basis areas inside the basis perimeter, the reattaching secretion seems to originate from old primary cement duct ends (Fig. 13). This reuse of old ducts is demonstrated in whole-mounts of the basis where several layers of additional secretion lie around the old duct ends (Fig. 14). The layered appearance of the secretion indicates that there was enough time for one layer to harden before the next one was laid down. Since in the course of normal development, the edge of the growing basis is pressed tightly on the substratum and the cement is spread in a very thin layer, these thicker layers of secretion must have appeared subsequent to the growth period during which the primary secretion took place. Therefore, we consider these thicker layers to be secondary secretions or secondary cement.

FIGURE 5. Microtome section through *Balanus crenatus* basis area showing three layers of striated secretions (10- μ section, Mallory's Trichrome, 220 $\times$).

FIGURE 6. Cluster of *Balanus crenatus* barnacles. Several faster growing specimens have lifted up a slower growing one, which continued to thrive in its elevated position.



FIG. 7

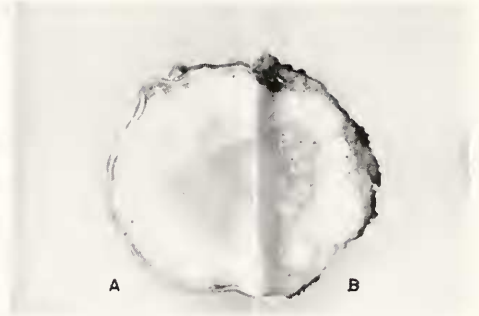


FIG. 10

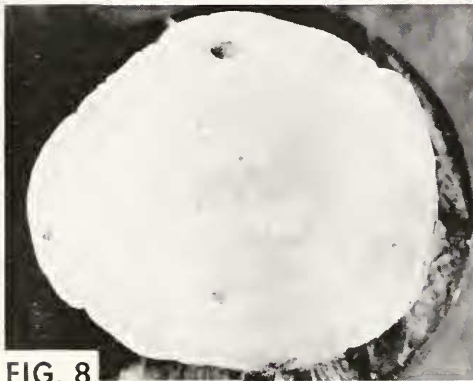


FIG. 8

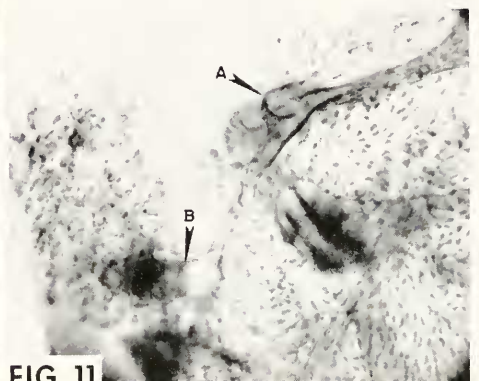


FIG. 11



FIG. 9



FIG. 12

FIGURE 7. Abundant mass of secretions on basis of *Balanus crenatus* specimen that was naturally detached by other barnacles.

FIGURE 8. Basis of *Balanus crenatus* after removal from smooth test panel in the laboratory.

FIGURE 9. Basis of specimen in Figure 8 after it reattached to a glass microscope slide in the laboratory.

FIGURE 10. Reattached *Balanus crenatus* specimen. Side A is firmly reattached to a glass microscope slide, while side B is left suspended. Drops of secretion hang from the basis on the side where the barnacle was unable to reach and adhere to the substratum.

The quantity of this secondary cement varies according to the size of the animal and the extent of the detachment. It is difficult to estimate the volume of the secondary cement because of its loose, cavernous structure. The volume of the denser primary cement also varies widely according to such factors as the size, age, and growth rate of the barnacle, and the surface characteristics of the substratum. The size and behavior of the very young adult, however, is more uniform. The first adult cement is secreted around the basis perimeter of the first order adult, which metamorphoses from the cyprid. The first order cement ducts are 170 microns long on the average and often not more than two microns in diameter in *Balanus crenatus*. The cement is spread around the perimeter of the basis of the young adult forming an elliptical ring with the following average measurements:

$$\begin{array}{ll} \text{outside semiaxes } A = 3(10^{-2}) \text{ cm} \\ \phantom{\text{outside semiaxes }} B = 4(10^{-2}) \text{ cm} \\ \text{inside semiaxes } a = 2.5(10^{-2}) \text{ cm} \\ \phantom{\text{inside semiaxes }} b = 3.5(10^{-2}) \text{ cm} \\ \text{thickness } d = 5(10^{-4}) \text{ cm} \end{array}$$

The average volume of the cement secreted by the first order adult *Balanus crenatus* is:

$$V = \pi (AB - ab)d = 5(10^{-7}) \text{ cm}^3$$

DISCUSSION

It was shown that in most cases an injured or detached barnacle uses the old cement duct system for repair or reattachment. This reuse of old ducts is possible only if the duct system connected to these areas is still functional and the passages are still open. Since the ducts were once filled with primary cement, it would seem that when the cement hardens, the ducts would be plugged with solidified material and thereby rendered useless for subsequent secretion. Darwin (1854), however, found that in *Balanus tintinnabulum* the ducts were free of cement. In *Balanus crenatus*, *B. glandula* and *B. nubilus* we found that this was only partially true. We often observed in these species a few cement ducts and vesicles full of hardened cement among the majority of empty ducts. (Fig. 15). It is impossible to offer any positive explanation for the cement-free status of the ducts without a thorough understanding of the hardening mechanism of the barnacle cement. At this time, however, the available information about the chemistry of the barnacle cement is very limited, therefore, we can resort only to theorizations.

Based on fluid mechanical considerations, it can be demonstrated that perhaps the most important physical requirement for the cement at the time of the secretion is low viscosity. According to the Poiseuille law, the pressure drop of a fluid flowing through a duct is directly proportional to the viscosity and the velocity of

FIGURE 11. New, irregular duct growing directly from a (A) vesicle into a (B) damaged area (Wholemout, TriPARS, 180 $\times$).

FIGURE 12. Growth of new, irregularly-shaped ducts originating from (A) vesicles into the (B) damaged initial attachment area (Wholemout, TriPARS, 70 $\times$).

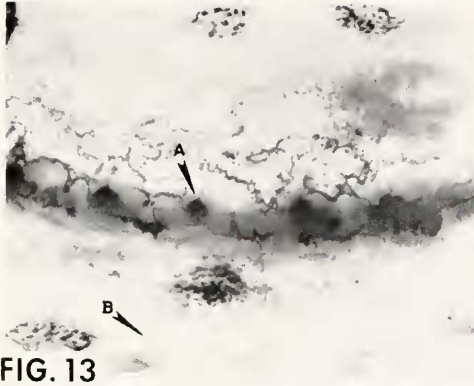


FIG. 13

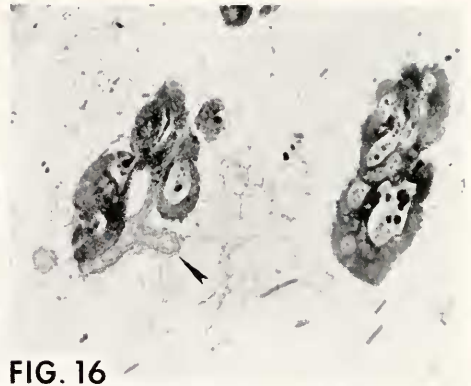


FIG. 16

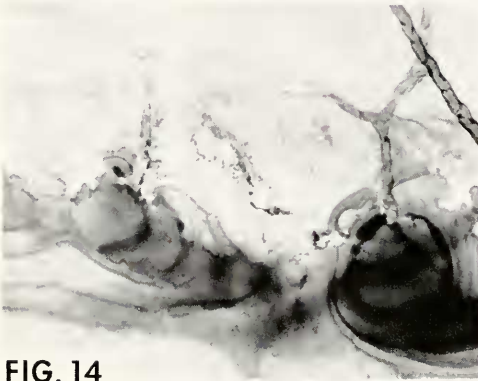


FIG. 14

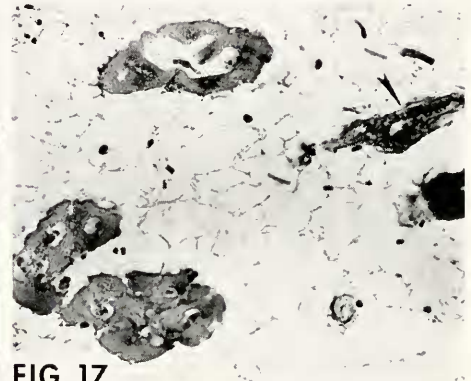


FIG. 17

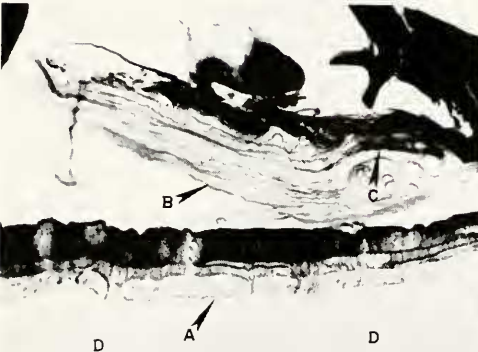


FIG. 15

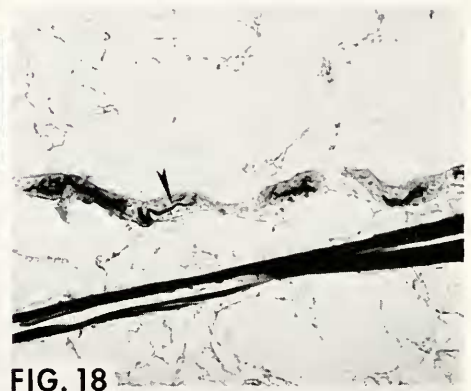


FIG. 18

FIGURE 13. Abundant secondary secretions lie around (A) old duct ends, which now contain hardened cement, at the site of a partial detachment of the basis. Newer (B) ducts, which were unaffected by the accident, are empty (Wholemout, Mallory's Trichrome, 70 $\times$).

FIGURE 14. Old duct ends with new, thick circular layers of cement around them (Wholemout, TriPARS, 180 $\times$).

FIGURE 15. Microtome section through basis area of *Balanus crenatus* that was attached to paraffin shows: (A) Thick irregular layers of cement secretions, (B) empty ducts, (C) duct containing cement, and (D) substratum (10- μ section, Mallory's Trichrome, 180 $\times$).

the fluid, and the length of the duct and inversely proportional to the fourth power of the radius of the duct.

$$\Delta p = \frac{8\eta vl}{\pi r^4}$$

where Δp = difference in pressure at the duct ends, η = absolute viscosity of fluid, v = volume of fluid delivered per unit time, l = length of duct, and r = radius of duct.

It is not yet known how long it takes for the barnacle to secrete the cement, but in a series of yet unpublished experiments, we found already hardened cement within 15 minutes after reattachment. For the sake of the present consideration, permit us to assume that the time interval for the secretion is about one tenth of the hardening time, or $t = 100$ sec. This would mean that the velocity of the secretion or the volume of cement delivered in a second by one of the first order ducts is:

$$v = \frac{\frac{1}{2}}{t} = 2.5(10^{-9}) \text{ cm}^3 \text{ sec}^{-1}$$

therefore, $\Delta p \approx 10^6 \eta$ (dyne cm^{-2} , if η is in poises).

In our example, the numerical value of the additional pressure in dyne cm^{-2} required to deliver the cement at the arbitrarily selected rate through the narrow ducts is about one million times the viscosity of the cement expressed in poises.

If the cement would have a low viscosity near that of water ($\sim 10^{-2}$ poise), the additional pressure would be only: $\Delta p = 10^4$ dyne cm^{-2} (or 0.145 psi). But, if the cement had a viscosity more like glycerol or castor oil (~ 10 poise), $\Delta p = 10^7$ dyne cm^{-2} (or 145 psi).

These considerations strongly suggest that initially the cement is a fluid of low viscosity which solidifies rapidly after secretion. Preliminary analysis of the hardened cement (Lindner and Dooley, 1969) indicates that the cement consists of mainly organic material and is probably a highly cross-linked polymer. The hardening mechanism of the cement therefore is very likely to be due to a polymerization of monomers (Saroyan, Lindner, Dooley and Bleile, 1970). To

FIGURE 16. The cement precursors are secreted by large, unicellular glands. As more of these glands develop with growth of the barnacle, they are joined to the others by collecting channels, indicated by the arrow, which transport the secretion from each individual gland to the node. The content of the collecting channels does not stain with Mallory's Trichrome (10μ section, Mallory's Trichrome, $180\times$).

FIGURE 17. The arrow indicates the node where the polymerization is believed to be initiated, because from this point on the channels stain red with Mallory. The red coloration seems to diffuse into the channel from the cytoplasm of the cells composing the node (10μ section, Mallory's Trichrome, $180\times$).

FIGURE 18. The activated cement, which stains intensely red with Mallory, then passes on through the main channel, shown by the arrow, which carries the cement from its polymerization initiation point to sites of its further distribution (10μ section, Mallory's Trichrome, $180\times$).

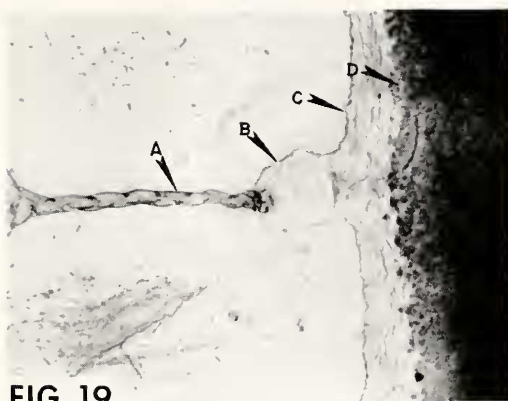
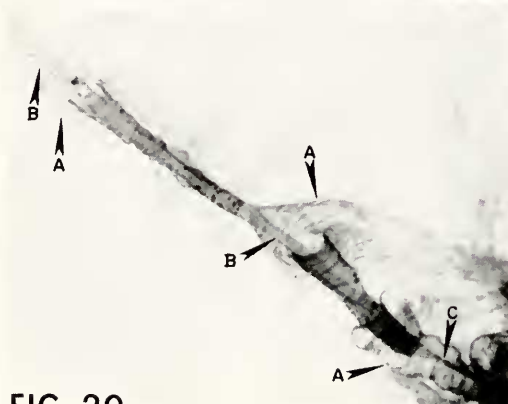
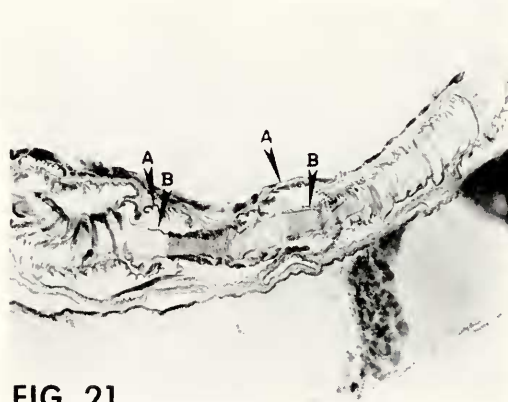
**FIG. 19****FIG. 20****FIG. 21**

FIGURE 19. Perimeter of an inner basis circle with (A) empty duct and (B) funnel-shaped orifice leading to the perimeter. The flushing process, following the cement secretion, has washed both the duct area and (C) a circular ring around the edge of the basis free of (D) cement before hardening took place (Wholemound, Mallory's Trichrome, 220 $\times$).

understand the cementing mechanism it would be of importance to know exactly how and where this polymerization is initiated.

There are indications that the cement polymerization is initiated after the secretion has left the cement glands and before it enters the duct network. Those observations (Saroyan, Lindner, and Dooley, 1968) that substances with staining characteristics similar to that of the hardened cement can be found in the main channel, but never in the secretion collecting side channels, and that the chemical characteristics of the secretion appear to be changed abruptly at the joining node of the collecting channels and the main channel suggest polymerization initiation at the node (Figs. 16, 17, and 18).

Initiation from the environment, namely the seawater, can be ruled out, since it was demonstrated that detached barnacles are also capable of reattachment with their bases and the new substratum out of the seawater. Occasional ducts or vesicles containing hardened cement also make the role of environmental factors questionable, since it is difficult to see how substances from outside could flow back against the cement current through the narrow ducts all the way to the vesicles and mix with the cement to harden it. For the same reason, secretions from the shell walls or elsewhere in the organism, but outside the ducts, ion exchange or pH change outside the orifices are unlikely to play an important role in cement hardening.

Initiation inside the ducts, for instance through the duct walls would not offer sufficient basis to explain the presence of cement-like substances further up in the main channel. Also, portions of secretion passing through shorter or longer ducts would be in contact with the initiating surfaces for a shorter or longer time, thus resulting in unevenly activated cement. It is therefore more likely that the polymerization initiation is restricted to a small portion of the conduits. The node, where the secretion from the scattered cement glands merges into one main channel on each side, is a seemingly ideal site for this.

Using this initiation mechanism, the cement is capable of hardening inside the ducts or vesicles. In order to explain why the majority of the ducts are free of hardened cement, we have to consider some mechanism capable of removing the activated secretion from the ducts either after or before the hardening of the cement is completed.

After the hardening of the cement is completed, the most probable method of its removal would be an enzymatic dissolution or refluidization of the cement, followed by the retention of the altered cement or its absorption through the duct walls. In either case, it is expected that new ducts would be found still containing hardened cement and no cement would be found in the older ducts, since the cement should have been removed from the latter long before. However, we found

FIGURE 20. (A) Vesicles with (B) main channel passing through them. (C) Collapsed main channel portion acting as a checkvalve to prevent vesicle content from backing up. (Wholemount, Gallego-Garcia, 220 $\times$).

FIGURE 21. Section through basis of an adult *Balanus crenatus* showing (A) the vesicles and (B) the main channel which passes through them (12- μ section, Mallory's Trichrome, 200 $\times$).

that all the cement-containing ducts have been older ones, originating in an earlier growing period. Therefore, there is little evidence to support this theory of removal.

Before the hardening is completed, the already activated cement, while it is still in the early stages of polymerization, can be flushed out and replaced by some nonhardening fluid. The most likely flushing fluid may be the non-activated cement containing its monomers but lacking its catalysts, or deactivated cement containing polymerization inhibitors, or modified cement containing non-reacting derivatives of its monomers. The polymerization can be initiated in two general ways, either "spontaneously" or "externally." Spontaneous polymerization is due to an inherent internal property of the system, in which all the necessary optimum conditions are present. External initiation requires the alteration of an existing condition, such as influencing the energetics, reactivity, or charge distribution of the system, or by adding a polymerization agent or subtracting an inhibitor.

Indications are that the polymerization is initiated externally rather than spontaneously. As was pointed out, the hardening seems to be initiated at the node because no trace of substances with cement-like staining characteristics were found in the collecting side channels, which conduct the secretion from the individual gland cells to the node, but from there on, the main channel invariably contains cement-like traces (Saroyan, Lindner, and Dooley, 1968). The polymerization therefore does not seem to be an inherent property of the secretion of the cement gland cells, but rather externally induced at the node by the mixing of different types of secretions from different types of gland cells or from the node itself by any of the above mentioned mechanism.

In view of these considerations, the cementing process starts in the mantle at the cement gland cells with the secretion of the inactive cement precursor, probably a monomer mixture or solution of low viscosity. The secreted precursor is collected by side channels and directed into the node. Here the secretion becomes activated, possibly by mixing with other secreted substances. The activated mixture merges into the main channel which conducts it from the mantle along the lateral scutal depressor muscle to the basis, where it enters the newest vesicle. From this vesicle the cement is distributed throughout the newly developed duct network and orifices around the enlarged perimeter. The cementing period ends with the secretion of the flushing fluid which displaces the still liquid cement from the ducts. In the course of normal development, this non-hardening flushing substance fills all the ducts, forces the cement out beyond the duct orifices and away from the edges of the basis. The cement hardens in this position under the cuticle of the joint connecting basis and shell walls. In this manner, a ten to forty micron wide circular channel is formed between the edge of the basis and the hardened cement, leaving the flushing fluid contained within this seal (Fig. 19).

After the normal cementing period the vesicle ceases to serve as a fluid transporting pump, in conjunction with the main channel, but still acts as an efficient checkvalve that prevents the flushing fluid from backing up and mixing with the new cement. Also, the structure of the vesicle enables it to control the flow of fluids and direct the new cement to where it is needed in the case of injury or detachment.

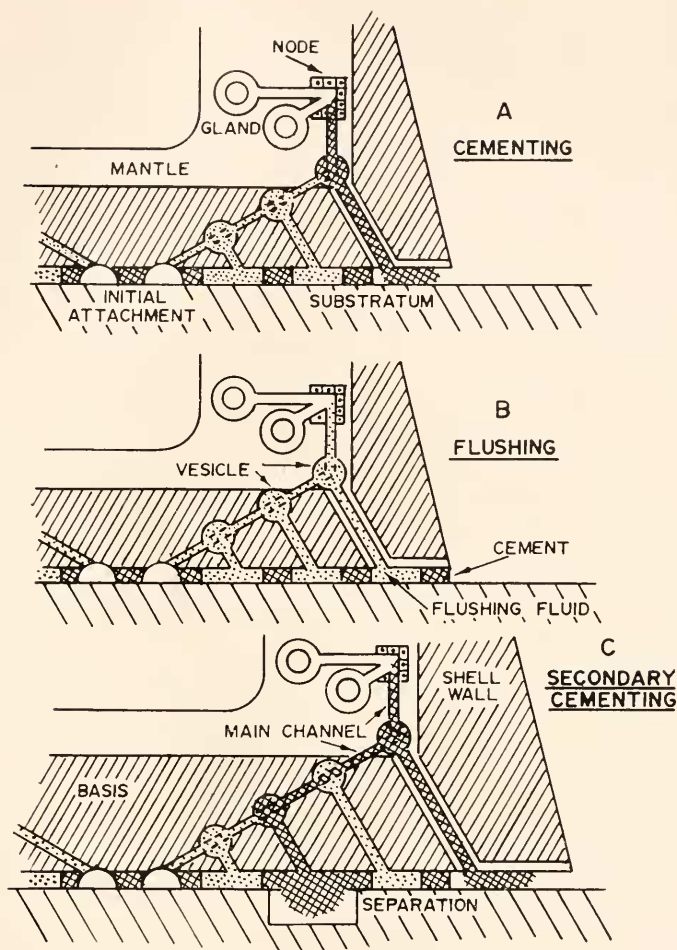


FIGURE 22. A Secretion of cement from cement gland to newest vesicle and duct network during normal development; B. Flushing of duct network following cement secretion; C. Secretion of cement when a separation from substratum has occurred in the region of an old duct.

As was pointed out, the main channel goes through the vesicles as what appears to be a continuous tube rather than as a simple connection (Figs. 20 and 21). That portion of the main channel which is inside the vesicle is probably permeable, permitting transflux into the vesicle, but largely reducing convection and diffusion between the contents of the main channel and the vesicle. As long as the flushing fluid fills the ducts and vesicles, the system remains in balance and no liquid passes through the permeable walls. In the course of normal development, therefore, the new cement does not go beyond the newest vesicle because the rest of the main channel and duct network is filled with the flushing fluid and no room is available for the cement (Fig. 22A). The new cement can enter only the new vesicle and

duct network to be secreted at the perimeter. The still liquid cement is displaced by the non-hardening flushing fluid at the end of the cementing period. The cement hardens outside the orifice and seals the flushing fluid inside the ducts (Fig. 22B).

However, if the basis separates from the substratum, the cement seal of some duct ends breaks and the flushing fluid drains out of the corresponding ducts and vesicles. In the same fashion, a fracture in the basis would sever some ducts and the flushing fluid would also leak out at the injured site. Due to this leakage, the balance of the system is upset. The pressure then drops on the leaking side of the permeable walls, and the loss of flushing fluid makes room for newly secreted cement. The cement is now allowed to pass further down the main channel until it reaches the vesicle affected by the injury and to pass through the permeable membrane of the main channel. The cement enters the vesicle and into the ducts of the injured network and follows the draining flushing fluid to the site of the injury or detachment (Fig. 22C). Since the duct networks of different growing periods are completely isolated from each other except at the vesicles, which are connected only by the main channel, the flushing fluid drains only from duct ends which are affected by the injury and only from those ducts which represent the shortest route between the injury and the corresponding vesicle. Other ducts, unconnected with the injured area, remain filled with the flushing fluid and, hence, the cement bypasses those vesicles which serve the unaffected duct network.

Thus, the vesicle, in conjunction with the main channel not only distributes and transports the cement by means of a pumping action, but also controls and regulates the flow of cement and flushing fluid, as would a valve. The vesicles of different age are situated near each other and are connected by the main channel. This arrangement puts all the vesicles, the corresponding duct networks, and especially the duct ends—regardless of the network to which they belong—almost equidistant from the cement glands. Thus, it is practically as easy to secrete cement through older ducts as through the newest, peripheral duct system. After emergency use, the network is again flushed out and ready for further reuse. In repeated use, however, the flushing process is not always complete because usually larger and irregular amounts of cement are secreted at the emergency locations, thus plugging the orifices. Hence, those reused ducts and vesicles could contain hardened cement.

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SUMMARY

Barnacles, which become partially or totally detached from their substratum in a natural environment, produce a secondary cement secretion. Laboratory experiments demonstrate that the secondary cement can successfully reattach the barnacle to a new substratum. Similar secondary secretion was found at the site

of minor injuries to the barnacle basis. The secondary cement usually has a looser, more cavernous structure than the primary cement, but both secretions have similar staining characteristics.

Microscope preparations indicate that occasionally barnacles are capable of developing new secondary cement ducts leading into the injured or detached areas to secrete secondary cement.

In most cases, however, the existing primary cement duct network is used for the secondary secretion. This is possible only because most of the once used ducts are not plugged by hardened cement, in spite of the fact that the cement can harden inside the ducts. Chemical analysis suggests that the cement is an organic biopolymer and indications are that the cement hardening is initiated inside the organism.

A unique flushing mechanism seems to be responsible for keeping the cement ducts open and ready for reuse. A nonhardening flushing fluid forces the still liquid cement out of the ducts. The cement hardens outside the duct openings sealing the flushing fluid inside the duct network. In case of detachment or injury, the cement seal breaks; the flushing fluid drains out leaving the duct open for the secondary cement secretion.

The vesicles in conjunction with the main channel control the flow of the flushing fluid and the cement. The permeable wall of the main channel portion inside the vesicle reduces the convection and diffusion between the vesicle and the main channel, thus bypassing of vesicles and duct networks not affected by detachment is possible. The wall of the main channel inside the vesicle is also collapsible, thus acting as checkvalve when the vesicle is under pressure and allowing the cement to be pumped only into the ducts toward the secretory orifices.

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